REPORTS

Long-Term Therapy With Low-Dose Isotretinoin for Prevention of Basal Cell Carcinoma: A Multicenter Clinical Trial

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Background: **High-dose isotretinoin has been reported to have a prophylactic effect on nonmelanoma skin cancer, although it is associated with significant toxicity.** ***Purpose:*** **To test the effectiveness of the long-term administration of low-dose isotretinoin in reducing the occurrence of basal cell carcinoma at a new site in patients with previously treated basal cell carcinomas and to measure the toxicity associated with this regimen, we conducted a clinical trial at eight cancer centers.** ***Methods:*** **Nine hundred and eighty-one patients with two or more previously confirmed basal cell carcinomas were randomly assigned to receive either 10 mg of isotretinoin or a placebo daily. Patients were followed for 36 months and monitored at 6-month intervals for skin cancer and toxic effects.** ***Results:*** **After 36 months of treatment, no statistically significant difference in either the cumulative percent of patients with an occurrence of basal cell carcinoma at a new site or the annual rate of basal cell carcinoma formation existed between patients receiving isotretinoin and those receiving the placebo. Elevated serum triglycerides, hyperostotic axial skeletal changes, and mucocutaneous reactions were more frequent in the group receiving isotretinoin than in the control group, and these differences were all statistically significant (*$P<.001$*).** ***Conclusion:*** **This low-dose regimen of isotretinoin not only is ineffective in reducing the occurrence of basal cell carcinoma at new sites in patients with two or more previously treated basal cell carcinomas but also is associated with significant adverse systemic effects.** ***Implication:*** **The toxicity associated with the long-term administration of isotretinoin, even at the low dose used in this trial, must be weighed in planning future prevention trials. [J Natl Cancer Inst 84:328–332, 1992]**

Basal cell carcinoma, a skin tumor of epithelial origin (*1,2*), is responsible for considerable morbidity and is expensive to treat. Each year, an estimated 600 000 new cases of nonmelanoma skin cancer develop, 80% of which are basal cell carcinomas (*3,4*). The predominant treatment for previously untreated basal cell carcinoma is surgical excision or ablation by other techniques, yielding cure rates of 95% or higher (*3*). However, the risk of development of new basal cell carcinomas in previously treated individuals can be very high (*5-7*).

Isotretinoin (13-*cis*-retinoic acid) at high dosage (2 mg/kg per day) was shown to have limited efficacy in the treatment of basal cell carcinoma (*8*). In a case series reported by Peck et al. (*8*), the therapeutic effect of high-dose isotretinoin given as a single agent was not striking, with observed regression occurring in nine of 65 basal cell carcinomas. Of note, however, was the fact that no new occurrences of the carcinoma developed at a different site in these high-risk patients over a 4-year period of treatment (*9*).

Because of isotretinoin's potential prophylactic effect on basal cell carcinomas, we decided to evaluate the effectiveness of the long-term administration of low-dose isotretinoin in preventing the occurrence of the first additional basal cell carcinoma at a different site in patients with a previously treated basal cell carcinoma and to determine the extent of the toxicity associated with this long-term, low-dose regimen. In choosing the optimal dose to be used in this trial, we considered isotretinoin's biologic activity, patient acceptance of treatment (given the drug's long-term dosing schedule and expected side effects), and the possible unmasking of

Received July 5, 1991; revised December 16, 1991; accepted December 16, 1991.

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We thank Dr. Ray Kilcoyne, Dr. Jere Stern, Janet Morgan, Dr. Earl Gross, and Oscar Chavez for their important contributions to the successful implementation of this trial.

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the trial by isotretinoin's characteristic mucocutaneous effects. The dose (10 mg per patient per day or approximately 0.14 mg/kg per day) was based on clinical information available at that time from Hoffmann-La Roche, Inc. (Nutley, N.J.) and a clinical study that showed that isotretinoin exhibited biologic activity at doses as low as 0.1 mg/kg per day (*10*). This report gives the results of a clinical trial evaluating 3 years of intervention with this regimen.

Patients and Methods

A detailed description of the trial's design, organization, and the baseline characteristics of the participants has been published (*11*). The study was a randomized, double-masked, placebo-controlled trial coordinated by the National Cancer Institute (Bethesda, Md.) and conducted at eight cancer centers in the United States. To be eligible to participate in the trial, a patient had to be a White male or White female between the ages of 40 and 75 years, had to have had two or more biopsy-proven basal cell carcinomas during the 5 years before being randomly assigned to a study group, had to have normal liver and renal functions, had to give written informed consent, had to be willing and able to participate for the duration of the trial, had to have entire skin surface evaluable for presence of basal cell carcinoma, had to agree not to take high-dose vitamin A (>5000 U/day), and, for women, had to be incapable of childbearing. Because the risk of developing subsequent basal cell carcinomas is directly related to the prior occurrence of basal cell carcinoma, the selection of patients with previous lesions allowed us to maintain a realistic sample size while ensuring an adequate number of study end points.

The accrual phase of the study, during which 981 patients were enrolled, started in February 1984 and closed in June 1987. Overall, one in five patients contacted (either through a clinic visit, questionnaire, or telephone) was ultimately enrolled in the trial (*11*).

After a screening visit to determine eligibility and to obtain written informed consent, patients were randomly assigned to receive either 10 mg isotretinoin (as two 5-mg capsules once daily) or a matching placebo (both supplied by Hoffmann-La Roche, Inc.). To monitor for skin cancer, potential treatment toxicity, and compliance (measured by pill count and interview), trial participants were scheduled for follow-up clinic visits at 2 weeks, 3 months, 6 months, and every 6 months thereafter for the 3-year duration of the intervention.

All putative adverse events defined as unpleasant or harmful effects, either temporary or permanent, experienced by the patients were recorded during the trial. If necessary, dose modifications were prescribed according to a standard protocol and documented at each follow-up visit until the adverse event ceased (*12*). All participants had baseline cervical and thoracic lateral radiographs at the time of initial enrollment and at the 36-month visit using methods described in previous studies (*13,14*). Potential axial skeletal changes were monitored by randomly selecting 269 patients (isotretinoin group, 139; placebo group, 130) for comparative review of their radiographs. These radiographs were read by a single board-certified radiologist with special interest and training in skeletal radiology as well as previous experience in examining x rays for retinoid-induced skeletal changes (R. Kilcoyne). The radiologist, from whom knowledge of the treatment assignment was withheld, completed the review in two sessions. Baseline and 36-month radiographs for each patient were read side by side (to allow comparison), with the radiologist noting both the presence and extent of baseline abnormalities at each vertebral level (C1 through T12) and any new occurrence or progression of existing abnormalities at 36 months. Only those findings that were considered by the radiologist to be extensive enough to be unequivocal were recorded.

Microscopic slides of all biopsy specimens were sent to the Data Coordinating Center for confirmation of local diagnosis by a central study dermatopathologist (J. Stern). A total of 8492 biopsy specimens were taken over the 3-year treatment phase of the study. Agreement between local pathology diagnoses and central diagnoses exceeded 90% during this period. Disagreement was resolved through review by a dermatopathologist at the Armed Forces Institute of Pathology, Washington, D.C.

The primary statistical analysis was a comparison of the cumulative incidence curves (where incidence was operationally defined as the first occurrence of a basal cell carcinoma at a new site) for patients taking isotretinoin (10 mg/day) and patients taking placebo over the 3-year treatment period. Analysis was performed using the two-sample, stratified logrank statistic (*15*) adjusted by specific clinic. Cumulative incidence estimates were calculated by the Kaplan-Meier method (*16*). Since basal cell carcinoma is rarely confined to one occurrence, the effect of isotretinoin on the multiplicity of basal cell carcinoma was measured by comparing the annual rate of tumor formation (defined as the total number of basal cell carcinomas for all visits divided by the number of person-years of follow-up) using a permutation test (*17*). Other two-group comparisons for evaluating adverse reactions required various standard parametric and nonparametric test statistics (*18,19*). Reported P values are two sided. All patients were included in the analyses, according to the principle of intent to treat.

Results

Baseline characteristics of the patients in each treatment group are presented in Table 1. There were no statistically significant treatment group differences for any of the characteristics listed. The mean age of the trial participants was 60.8 years, with males being slightly older than females. In the 5 years before trial entry, almost two thirds of the participants had had three or more confirmed basal cell carcimomas. Most trial participants were fair-skinned (Fitzpatrick skin grades 1-2) (*20*) and exhibited moderate to severe actinic damage at baseline.

Only about 8% of the patient population (8.7% isotretinoin group; 7.7% placebo group) were lost to follow-up over the 3-year treatment period, with losses equally divided over each of the 3 years of treatment. The mean numbers of days of follow-up on protocol over the 3-year treatment period were similar in the two treatment groups (isotretinoin group, 1030 days [SD = 228 days]; placebo group, 1042 days [SD = 203 days]). On average, patients in the isotretinoin group spent 89% of protocol follow-up time on

Table 1. Baseline characteristics of study population

Baseline characteristic	No. (%) Isotretinoin (n = 490)	Placebo (n = 491)
Sex		
Male	367 (74.9)	390 (79.4)
Female	123 (25.1)	101 (20.6)
Age, y		
<60	178 (36.3)	172 (35.0)
60-64	124 (25.3)	120 (24.5)
≥65	188 (38.4)	199 (40.5)
Previous basal cell carcinoma		
2	183 (37.4)	193 (39.3)
3-4	167 (34.1)	166 (33.8)
>4	140 (28.6)	132 (26.9)
Overall solar damage		
Mild	166 (34.2)	150 (30.8)
Moderate to severe	319 (65.8)	337 (69.2)
Skin type		
Fitzpatrick skin grades 1-2	309 (63.6)	294 (60.2)
Fitzpatrick skin grades 3-5	177 (36.4)	194 (39.8)

study capsules (913 days [SD = 345 days]), while patients in the placebo group spent 94% (981 days [SD = 284 days]) on capsules. Within the isotretinoin group, the theoretical mean cumulative isotretinoin dose for one patient taking two capsules daily over the 3-year treatment period was 10.95 g. Taking into account losses to follow-up, reduction in dosage to one capsule, or early discontinuation of study medication, patients in the isotretinoin group ingested, on average, 7.85 g of isotretinoin over a 3-year period.

In Fig. 1, the 3-year cumulative incidence curve for patients receiving isotretinoin is compared with that for patients receiving placebo. We observed no statistically significant difference between the two groups (P = .72). At the end of 3 years of intervention, 327 patients in the isotretinoin group and 327 patients in the placebo group had developed at least one new basal cell carcinoma. Isotretinoin also had no effect on basal cell carcinoma multiplicity, as measured by the annual rate of tumor formation. The tumor rate was 0.94 tumor per patient per year in the isotretinoin group, compared with 0.96 tumor per patient per year in the placebo group (P = 0.83). While cumulative incidence rates and annual rates of tumor formation were highest in males, in older patients, in patients with the greatest prior solar damage, and, particularly, in patients with the greatest number of prior occurrences of basal cell carcinoma in the 5 years prior to entering the study, there was no indication of a protective effect of isotretinoin in any of these subgroups (data not shown).

Table 2 shows the five major categories of adverse reactions reported and presents the number and percentage of patients who experienced each type of adverse reaction over the 36-month treatment period. Because patients could report more than one type of adverse reaction over the course of the trial, they may appear in more than one category. The number of patients who experienced elevated total serum triglycerides and mucocutaneous reactions over the 36-month treatment period was significantly higher in the isotretinoin group (P<.001 for both effects), while the number of patients with arthralgic/myalgic reactions was of borderline significance (P = .052). At 36 months, the mean level of total serum triglycerides was significantly higher in the isotretinoin group (mean for patients receiving isotretinoin = 1.63 mmol/L, and mean for patients receiving placebo = 1.45 mmol/L; P<.001), whereas the mean level of total serum cholesterol did not differ between the two groups (mean for patients receiving isotretinoin = 5.84 mmol/L, and mean for patients receiving placebo = 5.81 mmol/L; P = .34). The overall percentage of patients with adverse reactions in the isotretinoin group was 76%. However, 43% of the patients in the placebo group also experienced adverse reactions, primarily symptoms related to skin and mucous membrane drying (mucocutaneous) or muscular aches and pains (arthralgia/myalgia). Most adverse reactions reported in both groups were mild,

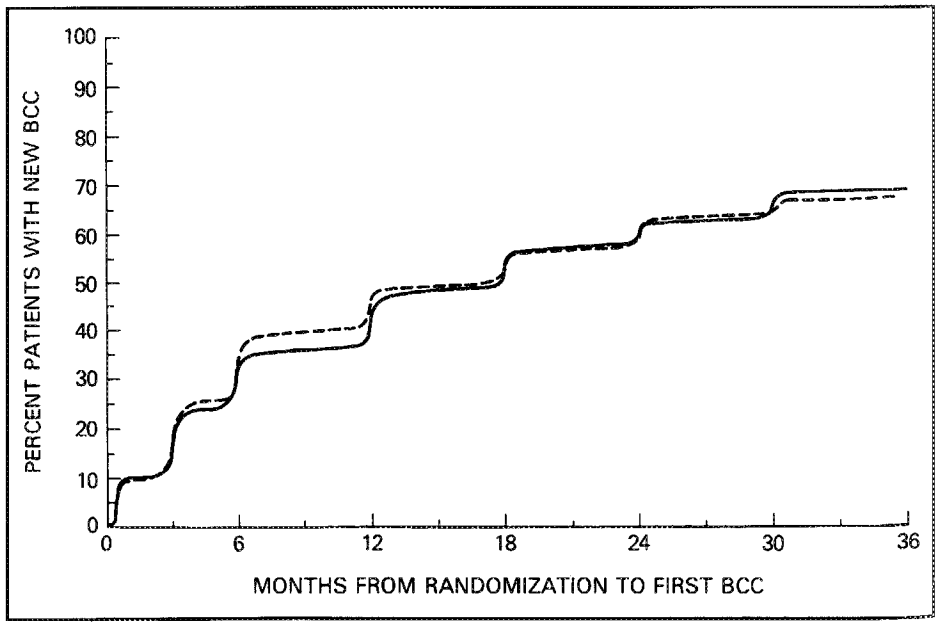

Fig. 1. Cumulative percent of patients with two or more previously treated basal cell carcinomas (BCCs) who had an occurence at a new site, according to treatment assignment. Solid line represents the group given isostretinoin (10 mg/d), and dashed line represents the group given placebo. The difference between the two groups was not statistically significant (P = .72).

and dose modification was considered necessary for less than 20% of the reactions.

The baseline (pretreatment) cervical and thoracic radiographs of the random sample of 269 patients reviewed showed a variety of abnormalities associated with aging, including extensive ankylosing vertebral hyperostoses, degenerative spondylosis, arthritic changes, and compression fractures. None of the patients whose radiographs were reviewed was free of abnormalities. Data on hyperostotic skeletal changes observed by radiograph between baseline and 36 months are presented in Table 3. These changes consisted of progression of pre-existing ossification of the anterior spinal ligament and/or increases in bony excrescences along the anterior-superior or anterior-inferior margins of vertebral bodies. In comparison with the patients receiving placebos, more than twice the number of patients receiving isotretinoin exhibited progression of cervical and thoracic hyperostotic vertebral abnormalities (56 patients or 40.3% of patients in the isotretinoin group versus 24 patients or 18.5% of patients in the placebo group; $P<.001$). Although fewer patients overall demonstrated new calcification or hyperostoses at previously unaffected vertebral levels, the number of patients with new hyperostotic vertebral involvement was also significantly higher in the isotretinoin group (12 patients or 8.6% of patients in the isotretinoin group versus two patients or 1.5% of patients in the placebo group; $P = .015$).

Discussion

In this randomized, placebo-controlled clinical trial, we found no difference between the isotretinoin group and the placebo group in the cumulative percent of patients with two or more previously treated basal cell carcinomas who experienced the first occurrence of the carcinoma at a new site. Similarly, we observed no reduction in the multiplicity of basal cell carcinomas in patients receiving isotretinoin compared with that in control patients, as measured by the annual rate of tumor formation. These findings contrast with those of one small non-randomized cross-over trial (*21*) and several case reports (*9,22-24*) using high-dose isotretinoin to prevent new occurrences of skin cancer in patients with previously diagnosed skin cancer. The present trial differs from these other studies in that it employed a randomized design, had a large sample size, and used a much lower dose of isotretinoin (one-half to one-fourteenth as much). This low dose may have contributed to the lack of efficacy observed. Patients selected for this trial had skin that was already substantially initiated and promoted given their prior history of skin cancer. It remains to be seen whether a low-dose, long-term regimen of isotretinoin might have an effect in other populations with less initiated skin, such as those with actinic damage but without skin cancer. Such a trial could raise serious ethical concerns, however, given the known toxicity profile of isotretinoin and the number of patients that would need to be entered to detect a therapeutic effect.

Indeed, even at the very-low-dose regimen used in this trial, the characteristic side effects of isotretinoin were manifested in the isotretinoin group. We also found that isotretinoin at the low dose used in this trial induced hyperostotic skeletal changes in the cervical and thoracic spine similar to those reported in patients taking higher doses (*25-32*). Radiographically, the changes observed were similar to the axial skeletal changes associated with naturally occurring, diffuse idiopathic skeletal hyperostosis (*33*) and appeared to be a continuation of a pre-existing process rather than a unique or distinct pathologic change. There were no significant treatment group differences in self-reports of back pain or stiffness over the treatment phase in the random sample of patients observed for radiographic changes. Since we did not perform systematic measures of flexibility or range-of-motion studies in these patients, we cannot rule out subtle clinical effects. However, the location and magnitude of these changes make them unlikely to be of clinical importance (*33,34*). Further radiographic monitoring will be performed to determine if the progression of skeletal changes seen in the isotretinoin group during the treatment phase of this study persists, worsens, or regresses after low-dose isotretinoin intervention ends.

Table 2. Number and percentage of patients with adverse reactions

Category	No. (%) Isotretinoin (n = 490)	No. (%) Placebo (n = 491)	P*
Elevated triglycerides†	36 (7.3)	8 (1.6)	<.001
Elevated liver enzymes‡	14 (2.9)	9 (1.8)	.289
Mucocutaneous§	344 (70.2)	173 (35.2)	<.001
Arthralgia/myalgia§	57 (11.6)	39 (7.9)	.052
Other‖	41 (8.4)	28 (5.7)	.103
Total	373 (76.1)	212 (43.2)	<.001

*Test for the difference between two proportions (z statistic).
†For serum triglycerides, fasting levels >3.39 mmol/L.
‡For serum aspartate aminotransferase and/or alanine aminotransferase levels at least 1.5 times greater than patient's baseline level and exceeding the upper limit of normal at the individual clinical center's laboratory.
§Includes symptoms elicited by questionnaire, detected on physical examination, or self-reported.
‖Includes miscellaneous subjective symptoms self-reported by patients (e.g., anxiety, fatigue, constipation, and dizziness).

Table 3. Number and percentage of patients with spinal ligament calcification and/or vertebral hyperostoses detected by radiography after 36 months of treatment

Type of change	No. (%) Isotretinoin (n = 139)	No. (%) Placebo (n = 130)	P*
Progression of existing calcification or hyperostoses	56 (40.3)	24 (18.5)	<.001
New calcification or hyperostoses†	12 (8.6)	2 (1.5)	.015

*Test for the difference between two proportions (chi-square statistic).
†Of previously unaffected vertebral level.

In contrast to a recent chemoprevention study, in which a high-dose regimen of isotretinoin (50-100 mg/m^2 per day or 1.3-2.6 mg/kg per day, based on a 70-kg, 170-cm patient) proved effective in reducing the rate of occurrence of second primary regional tumors in patients with previously treated squamous cell carcinomas of the head and neck (*35*), the low-dose regimen of isotretinoin used in the present study demonstrated no beneficial effect in patients with two or more previous occurrences of basal cell carcinoma who were at moderate-to-high risk of developing an additional basal cell carcinoma at a different site.

While efficacy was observed in the earlier study, the high dose of isotretinoin used by Hong et al. (*35*) resulted in significant toxic effects in the participants, with 33% of the subjects dropping out over the 12-month treatment period. Furthermore, skeletal changes were not monitored. Although the toxicity that occurred in our trial was much less than that reported by Hong et al. (*35*), it is clear that, even at the low-dose regimen used in the present study, isotretinoin has adverse systemic effects. Because chronic toxicity is a major consideration in the selection of cancer chemopreventive agents, the occurrence of measurable side effects even at this very low dose of isotretinoin must be weighed in planning future prevention trials.

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Delayed Tumor Onset in Transgenic Mice Fed a Low-Folate Diet

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Background: **Transgenic mice carrying the human T-lymphotropic virus type I tax_1 (transactivator) gene develop peripheral nerve sheath tumors with well-characterized times of onset and tissue involvement.** ***Purpose and Methods:*** **To evaluate the effect of dietary folic acid on age at tumor onset and on the concentration of folate in tissues and tumors, we bred heterozygous transgenic mice and systematically assigned their offspring at weaning (within litters) to a 2 × 2 × 2 factorial arrangement. The three variables studied were 1) the tax_1 gene (presence or absence), 2) gender (male or female), and 3) dietary level of folic acid (0.11 or 11.34 μmol folic acid per kilogram of controlled amino acid-based diet). Blood and tissues were col-**